

## **REMARKS/ARGUMENTS**

Upon entry of this response, claims 11 will be amended to correct an error of a typographical nature by changing computer to the clearly intended composition, whereby claims 9-14 will remain pending. Claims 9 and 12 are independent claims.

Reconsideration and allowance of the application are respectfully requested.

### **Information Disclosure Statements**

Applicants express appreciation for the Examiner's confirmation of consideration of Applicants' Information Disclosure Statement filed March 24, 2004 by including an initial copy of the Forms PTO-1449 submitted therewith.

### **Response To Rejections**

Claims 9-14 are rejected on the ground of non-statutory obviousness-type double patenting as being unpatentable over claims 1-8 of U.S. Patent No. 6,756,231 in view of U.S. Patent No. 6,201,134 to Nagano et al.

In response, Applicants respectfully submit that the rejection is without sufficient basis in that the rejection only indicates disclosure of the '134 patent and does not point to the '231 patent. Thus, the basis of this rejection is not clear, and without appropriate basis.

In any event, to advance prosecution of the application, and without expressing agreement or acquiescence with the rejection of record, Applicants are submitting herewith an executed Terminal Disclaimer. Accordingly, this ground of rejection should be withdrawn.

Claims 9-14 are rejected under 35 U.S.C. 102(b) as being anticipated by WO 99/01447 to Nagano et al. having a publication date of January 14, 1999, and its U.S. national stage family member 6,201,134 to Nagano et al., as an English translation of the International publication (and these documents will be collectively referred to hereinafter as "Nagano")

Applicants respectfully submit that Nagano does not clearly envisage, the instantly claimed subject matter to constitute anticipation. For example, Nagano does not specifically envisage Applicants' recited compounds recited in claims 9-11 having an amino group and a methyl amino group at the locations included in Applicants' recited compounds.

Regarding claims 12-14, Applicants respectfully submit that that Nagano does not clearly envisage, the instantly claimed subject matter to constitute anticipation. For example, Nagano does not specifically envisage Applicants' recited compounds having a methyl group as included in Applicants' recited compounds.

Thus, Nagano does not disclose compounds recited in Applicants' claims with sufficient specificity as to constitute anticipation, and the rejection should be withdrawn.

Applicants also remind the Examiner that Nagano is cited and discussed in Applicants' originally filed specification with advantages being provided over compounds included in the compounds disclosed by Nagano.

Applicants' specification, beginning at the bottom of page 3, discusses two methods for measuring nitric oxide.

One of the methods disclosed in Japanese Patent Unexamined Publication (Kokai) No. 10-226688/1998 utilizes a diaminofluorescein derivative (referred to as "DAF"). This

method utilizing DAF is disclosed as being excellent in reactivity with nitric oxide and measurement sensitivity, and as enabling measurement of nitric oxide with excitation light of a long wavelength that does not damage living tissues and cells, and accurate measurement of nitric oxide existing in inside of cells for each individual cell. However, it is disclosed that since a part of fluorescence wavelength range of the triazole derivatives (referred to as "DAF-T") that are produced by the reaction of DAF with nitric oxide overlaps with the autofluorescence range of cells, the method may sometimes fail to accurately measure nitric oxide in certain types of samples. Further, it is disclosed that since the fluorescence of DAF-T may be attenuated from weakly acidic to acidic region, a problem also arises in that accurate measurement over a wide pH range cannot be conducted.

The second method utilizing a diaminorhodamine derivative (referred to a "DAR") to measure nitric oxide is disclosed in WO99/01447. It is disclosed that this method is based on the measurement of fluorescence of a triazole derivative (referred to as "DAR-T") which is produced by the reaction of DAR with nitric oxide. It is disclosed that the peak of the fluorescence spectrum of DAR-T lies around 580 nm (excitation wavelength: 565 nm), while the peak of the fluorescence spectrum of the aforementioned DAF-T is observed around 515 nm (excitation wavelength: 495 nm). It is noted that measurements without being influenced by autofluorescence of cells can be performed by using DAR. Furthermore, it is disclosed that since DAR-T can maintain a certain level or higher intensity of fluorescence in an acidic region as well as in a basic or a neutral region, it enables measurement of nitric oxide over a wide pH range. However, it is also noted that in the method utilizing DAR, fluorescence intensity of some DAR-T may sometimes

slightly fluctuate depending on pH, and a problem arises that accurate measurement cannot be performed when a sample is measured whose pH is possibly fluctuate during measurement, e.g., a tissue of a patient with an ischemic disease. There is also a problem that DAR-T has lower fluorescence intensity as compared to the class of DAF-T.

Applicants' present invention provides compounds useful for the measurement of nitric oxide which have higher fluorescence intensity and whose fluorescent intensity is not fluctuated by pH, which compounds are based on DAR that can maintain a certain level of fluorescence intensity in an acidic region as well as in a basic and a neutral region without being influenced by the autofluorescence.

Superior results associated with Applicants' compounds as compared to compounds disclosed in Nagano are presented in Applicants' Examples, and illustrated in Figs. 1 and 2. Thus, Fig. 1 illustrates that DAR-MT according to the present invention provides a more constant fluorescence intensity with pH fluctuation as compared to DAR-1T and DAR-2T of Nagano. Moreover, Fig. 3 illustrates a higher sensitivity for DAR-M and DAR-4M according to the present invention as compared to DAR-1 and DAR-2 of Nagano.

In view of the above, Applicants respectfully submit that the rejection of record is without appropriate basis in that Nagano should not be considered to anticipate Applicants' claims, and the rejection should therefore be withdrawn.

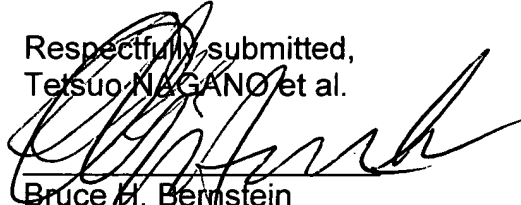
### **CONCLUSION**

In view of the foregoing, the Examiner is respectfully requested to reconsider and withdraw the rejections of record, and allow each of the pending claims.

Applicants therefore respectfully request that an early indication of allowance of the application be indicated by the mailing of the Notices of Allowance and Allowability.

Should the Examiner have any questions regarding this application, the Examiner is invited to contact the undersigned at the below-listed telephone number.

Respectfully submitted,  
Tetsuo NAGANO et al.



Bruce H. Bernstein  
Reg. No. 29,027

September 21, 2006  
GREENBLUM & BERNSTEIN, P.L.C.  
1950 Roland Clarke Place  
Reston, VA 20191  
(703) 716-1191

Arnold Turk  
Reg. No. 33094

Reg. No. 33094  
Arnold Turk